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GLYCOGEN INCREASE IN THE BRAIN AS A REACTION TO INJURY

With Report of Glycogen Studies in the Rat Brain Following Exposure to Ionizing Radiation

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It is well recognized that abnormal deposition of glycogen may occur in the brain under a variety of pathological conditions (Alzheimer, 1910; Casamajor, 1913; Helmke, 1937; Marinescu, 1928; Münzer, 1928). The deposition occurs in glial cells and may either be generalized or be found at the periphery of lesions when tissue damage has been severe (Friede, 1954; Shimizu and Hamuro, 1958).

The purpose of this report is to indicate that glycogen may also be deposited in the brain as a reaction to injury from ionizing radiation, both particulate and electromagnetic. In our experiments the brains of rats were exposed to (1) 12 Mev/nucleon alpha particles in a dose of 6,000 rad (surface dose) (30,000 rad depth dose) given at the rate of 10,000 rad/min, or 4×10^9 particles/cm^2/sec; (2) 10.4 Mev/nucleon alpha particles at surface doses ranging from 500 to 24,000 rad (depth doses 4 times those amounts) given at the rate of 24,000 rad/min, or 9.6×10^9

Dedicated to Professor Eufemiusz J. Herman on the occasion of the 45th anniversary of his scientific activity.

particles/cm^2/sec; and (3) 250 Kv X-rays at doses ranging from 500 to 28,000 r given at the rate of 100 r/min. Penetration of the brain by alpha particles was only to a depth of approximately 1.0 mm, and in the region of maximal penetration their energy release was, as indicated, 5 and 4 times, respectively, that at the brain surface. The principle involved here is that, as particle ~~energy~~ velocity decreases in passing through tissue, the rate of energy transfer and the absorbed dose increase, so that at the ionization peak (Bragg peak) in the region of termination of the particle beam the damage is most intense (Baker et al., 1961; Janssen et al., 1962; Rose et al., 1960; Tobias, 1962). The most damaged region, present at the depth of particle radiation, is referred to by us as the "Bragg-peak band," or, simply, "band." In the X-irradiated animals the midline tissue dose rate was estimated to be 89 rad/min.

Changes Following Alpha-Particle Irradiation. (a) With 12 Mev/nucleon alpha particles at 6,000 rad surface dose. Earlier studies indicated the presence, in the irradiated region, of aggregates of PAS-positive granules which were shown to contain glycogen, both histochemically* (Table 1) (Klatzo et al., 1961, 1962) and chemically (Wolfe et al., 1962). The glycogen granules, present chiefly in astroglia, were visible at 6 hours after irradiation, increased in number in time, with maximal number at 48 hours; they then gradually decreased until the 36th day, after which the few PAS-positive granules persisting were found free from glycogen

Table 1

*Rossman's fluid was used for tissue fixation: Absolute alcohol, 225 cc; picric acid, 22.5 gm; 40% formaldehyde, 25 cc.

Table 2 (Table 2). At earlier postirradiation stages, e.g., at 48 hours, glycogen granules were present above and below the band, not in the band itself

Fig. 1 (Fig. 1). In the studies utilizing chemical analysis, both the total and the trichloracetic-acid-extractable glycogen were found significantly increased (maximum of 23 and 50% increase, respectively) over a period of 8 days following irradiation.

(b) With 10.4 Mev/nucleon alpha particles at various doses, and with animal sacrifice at 48 hours. The lowest dose at which PAS-positive (glycogen-containing) granules were observed at the 48-hour period was 2,000 rad (surface dose) (Table 2). On the other hand, at 24,000 rad (surface dose), although striking damage had occurred in the irradiated region--so much so that myriad macrophages had formed--PAS-positive material had accumulated in the irradiated tissue and had been taken

Fig. 2 up by the macrophages (Fig. 2), but digestion analyses revealed no glycogen (Miquel et al., 1963a).

Changes Following 250 Kv X-Irradiation. (a) At various dose levels, with animal sacrifice at 24 hours. The lowest dose at which PAS-positive (glycogen-containing) granules were observed at 24 hours was ~~1,000 r~~ 1,200 r (Table 2). At succeedingly higher doses up to and including 8,500 r, they were progressively more numerous (Miquel et al., 1963b).

(b) At 300 and 3,000 r dose levels, with animal sacrifice at 5 to 96 hours. In the brains exposed to 300 r, no PAS-positive (glycogen-containing) granules were found over the time span indicated (Table 2). On the other hand, at 3,000 r they were found in all the brains, but their

number reached a peak at 48 hours (Miquel et al., 1963b).

Comment. These various studies, still in progress, indicate clearly that glycogen accumulates in the brain following exposure to ionizing radiation at certain dose levels. From presently available time-dose data it is clear that the granules increase in number with dose and that the peak of granule accumulation is at 48 hours postirradiation. Table 2 indicates the lowest radiation doses at which granules appeared at certain time periods, e.g., at 2,000 rad in 48 hours on exposure to 10.4 Mev/nucleon alpha particles. Whether granules will appear after a longer latent interval at doses under 2,000 rad still needs to be determined. The presently available data would seem to discount this possibility. Our studies with 12 Mev/nucleon alpha particles at the 6,000 rad surface-dose level have indicated that the appearance of PAS-positive glycogen granules represents a highly sensitive indicator of radiation injury at the dose levels ~~indicated~~ utilized. The sequence of changes observed under these conditions is indicated in Table 3, which contains data from various sources (Haymaker, 1962, 1963; Janssen et al., 1962; Klatzo et al., 1961, 1962).

Table 3

The mechanism by which glycogen increase occurs in the brain under irradiation conditions is still not clear. It has been suggested that protein bonds of tissue glycogen are disrupted by the radiation and that the glycogen freed in this manner is released from the injured tissue and taken up, through pinocytosis, by astroglia (Klatzo et al., 1961). On the basis that glycogen granules were not found in the Bragg-peak band

but were present both above and below the band (Fig. 1), and on the basis that at high X-ray doses glycogen increase did not occur (Fig. 2), it seems reasonable to assume that inactivation of an enzyme system, such as phosphorylase, may be concerned. A view which has been favored in that the granules may be ~~dryied~~ derived from carbohydrate substances liberated in the injured tissue and taken up by astroglia and converted to glycogen (Wolfe et al., 1962).

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TABLE 1

Stains and Procedures Used for Histochemical Analysis of PAS-Positive Granules

Stains and Procedures	Results
PAS Stain	Positive staining of the granules
Best's Carmine Stain	Positive staining of the granules
Acetylation (Gersh)	General reduction in PAS-positive staining
α-amylase	PAS-positive granules extracted
β-amylase	PAS-positive granules extracted
Hot chloroform-methanol 1:1	PAS-positive granules extracted
Metachromasia	Negative
Sudan Black B Stain	Negative

TABLE 2

Radiation Conditions Under Which Glycogen-Containing Granules Were Found in the Rat Brain

Radiation Conditions	Results
12 Mev/Nucleon Alpha Particles at 6,000 rad Surface Dose, with Animal Sacrifice from 5 Min to 36 Days (41 Animals)	Granules present at 6 hr, peak accumulation at 48 hr (histochemically) and 8 days (chemically), absent at 36 days
10.4 Mev/Nucleon Alpha Particles at 500, 1,000, 2,000 - 12,000 and 24,000 rad Surface Doses, with Animal Sacrifice at 48 Hr (28 Animals)	Granules present at 2,000, 6,000 and 12,000 rad, not at 500, 1,000 or 24,000 rad. Number of granules was roughly proportional to dose
250 Kv X-rays at 150, 300, 600, 1,200 - 8,500 r, with Animal Sacrifice at 24 Hr (58 Animals)	Granules present from 1,200 onward to 8,500 r. Number of granules was roughly proportional to dose
250 Kv X-rays at 300 and 3,00 r at time intervals from 5 to 96 Hr (24 Animals)	At 300 r, no granules were found over this time span. At 3,000 r, they were present over the entire time span, but their number reached a peak at 48 hr

TABLE 3

Time of Appearance of Changes on the Cerebral Cortex Following Exposure to 12 Mev/Nucleon Alpha-Particle Radiation at 6,000 rad Surface Dose

Nature of Change	Time of Appearance
Glycogen in Astroglia	6 hr
Nerve-Cell Damage	6 hr
Astroglial Damage (Cajal Technique)	48 hr
Vascular Alterations (Pickworth-Lepehne Technique)	48 hr
"Blood-Brain-Barrier" Penetration (by fluorescein-labeled albumin)	48 hr
Severe Myelin Damage	96 hr*

*Damage is assumed to have occurred at some period between 48 and 96 hours. No brains at the 72-hour postirradiation stage were available for study.

LEGENDS

Fig. 1. 12 Mev/nucleon alpha-particle radiation; 48 hours after exposure to 6,000 rad (surface dose). Cerebral cortex. A. The Bragg-peak band (the narrow pale strip with greatest nerve-cell loss) is virtually free from granules. Aggregates of glycogen granules are to be noted above and below the band. B. From corresponding section of the same brain after α-amylase digestion. The glycogen granules have disappeared. The Bragg-peak band is visible at the same level as in A. Both sections were stained by PAS and counterstained with hematoxylin. X 250.

Fig. 2. 10.4 Mev/nucleon alpha-particle radiation; 48 hours of the exposure to 24,000 rad (surface dose). Cerebral cortex, illustrating nerve-cell loss and macrophage response. PAS-positive material was found in the macrophages and in the irradiated tissue in general, but digestion procedures revealed no glycogen. The staining and digestion procedures were the same as in the section illustrated in Figure 1. X 100.

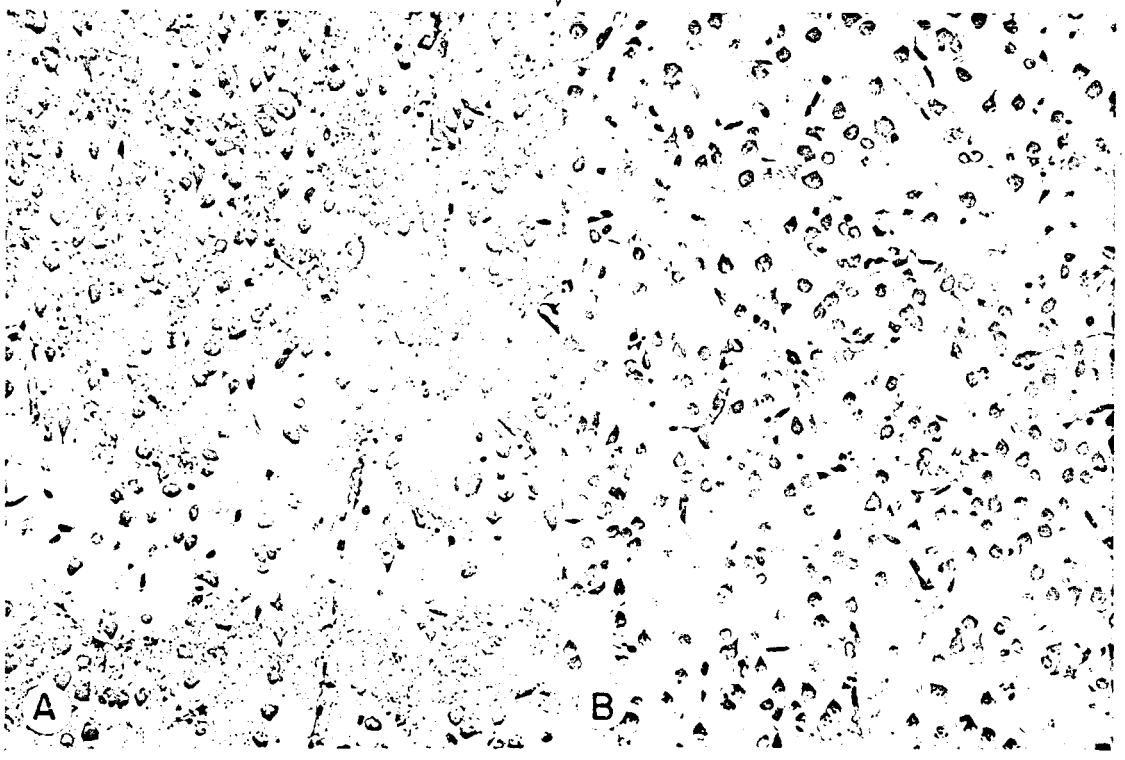

Fig. 1

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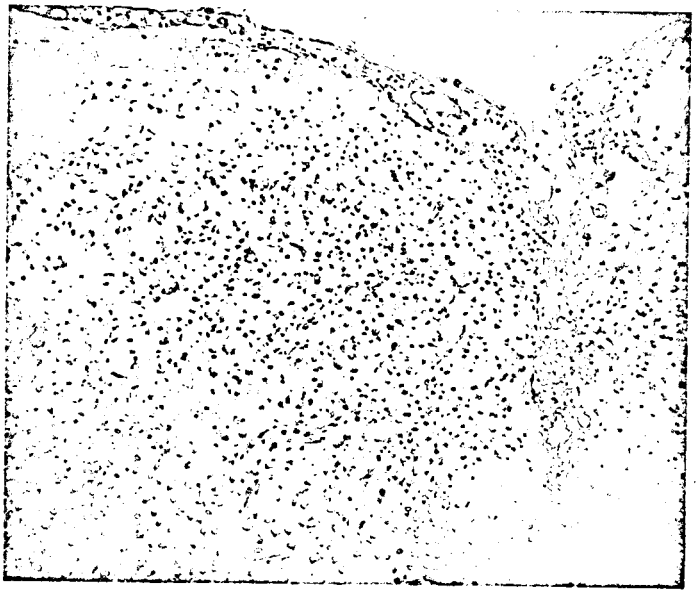

Fig. 2

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